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REVIEW

Immunological diagnosis of human angiostrongyliasis due to *Angiostrongylus cantonensis* (Nematoda: Angiostrongylidae)

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Summary *Angiostrongylus cantonensis*-associated eosinophilic meningitis in humans has been commonly reported worldwide. However parasitologically confirmed cases are not common, as the parasite has been recovered only infrequently from the cerebrospinal fluid of patients. The potential value of immunodiagnosis is therefore self-evident. Immunological tests can also help in the differential diagnosis of parasitic (particularly helminths) infections that cause eosinophilic meningitis. This paper summarizes the state of and advances in the immunological diagnosis of human angiostrongyliasis due to *Angiostrongylus* (= *Parastrongylus*) *cantonensis*. A specific antigen is available for the definitive diagnosis and unequivocal differentiation of eosinophilic meningitis due to helminth infections. Rapid diagnostic kits based on dot-blot ELISA have been developed and have proved to be simple, effective, and economical for field use.

Introduction

The rat lungworm *Angiostrongylus* (= *Parastrongylus*) *cantonensis* (Figure 1) is a food-borne zoonotic nematode parasite of considerable public health concern in many countries in the tropics and subtropics of both the Old and New Worlds.[1] In its life cycle it has a definitive rodent host and a mollusk intermediate host. The adult worms live in the pulmonary arteries of rats.

A. cantonensis is the causative agent of a form of eosinophilic meningitis (or meningoencephalitis) in humans, with marked cerebrospinal fluid (CSF) eosinophilia. The illness may persist for weeks or months. The treatment of eosinophilic meningitis due to helminths includes albendazole, steroids, and supportive care. Most cases are self-limited and resolve without complications. Neurologic sequelae do develop in some cases. The mortality of the disease is low, about 2—3%.

The human is a non-permissive, accidental host. Since about 1961, human infections have been known to be acquired by the ingestion of the infective third-stage larvae contained in raw or inadequately cooked food — either the intermediate mollusk hosts (snails and slugs) or animals

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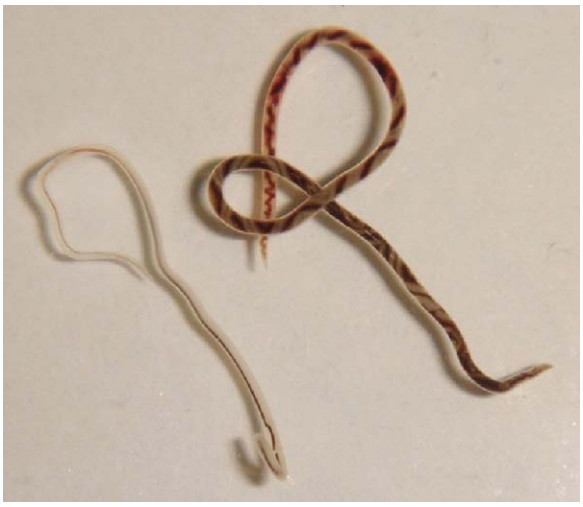

Figure 1 A*ngiostrongylus cantonensis* adult worms: left, male (body length 13—20 mm); right, female (body length 16—26 mm).

acting as a paratenic host (planarians, crustaceans, frogs, monitor lizards, etc.) — and contaminated fresh vegetables including raw vegetable juice.[1] The condition has been referred to as angiostrongyliasis cantonensis.

Diagnosis of human angiostrongyliasis cantonensis is based on clinical features as well as laboratory findings. In areas where infections are endemic, a presumptive diagnosis can usually be made on the basis of specific eating habits and symptoms of severe headache and eosinophilic meningitis or meningoencephalitis, with fever and ocular involvement.[1—3] Characteristically there is pleocytosis in the spinal fluid with the eosinophil count ranging from 26% to 75%, and the peripheral eosinophilia typically ranging from 5% to 63%.[2,4] Computed tomography (CT) scans and magnetic resonance imaging (MRI) techniques may reveal the presence of lesions in the brain and are useful for following up the disease or monitoring the complications, but they cannot serve as the basis for differential diagnosis.[5—8]

Although eosinophilic meningitis and meningoencephalitis may be indicative of the infection, the various clinical presentations caused by *A. cantonensis* must be differentiated from those caused by other tissue-migrating helminths, viz. *Gnathostoma* spp, *Paragonimus* spp, and *Taenia solium* metacestodes.[4,9,10] The suspected diagnosis can only be confirmed upon finding and identification of *A. cantonensis* larva(e) or young adult(s) in the CSF of patients. Although a large number of patients have been reported with the parasitosis, only a few cases have been confirmed by the finding of worms in the CSF.[1,4]

In an endemic area the epidemiologic and clinical features help in making a diagnosis. However eosinophilic meningitis may be due to infectious or noninfectious causes and therefore requires different regimens for effective management. Furthermore eosinophilia may not be present in the CSF or in the peripheral blood during the initial phase. A diagnosis using immunologic methods will therefore result in appropriate treatment and effective management of the disease.

During the last two to three decades, there have been at least two comprehensive reviews of the literature on immunological diagnosis of human angiostrongyliasis cantonensis.[11,12] Serological testing has now become widely accepted as the most appropriate diagnostic approach. Although earlier attempts to use ELISA, indirect hemagglutination (IHA), indirect immunofluorescent antibody test (IFAT), counterimmunoelectrophoresis (CIE), and the like have not been unequivocal, with more recent developments and refinement, immunological diagnosis will become an easy, cheap, and rapid technique.[13,14]

Antibody detection

The gold standard for the definitive diagnosis of angiostrongyliasis due to *A. cantonensis* is the finding of either the larva or juvenile worm in the CSF or in the eye chamber of an infected individual. Such a diagnosis is rarely achieved since worms are seldom found in the limited volume of the CSF obtained for diagnosis. This problem has prompted the development of immunological means for detection. An increase in antibody titer can be used as evidence of recent infection and also a likelihood of existing infection. The detection of antibodies encompasses a wide range of approaches and has received the most attention.

Over the past decades, a number of immunological tests have been developed to support the clinical diagnosis, in which crude somatic antigens or partially purified antigens of *A. cantonensis* adult worms, brain-stage larvae, or excretory—secretory products are most used.[15—42] ELISA has been most widely applied, and has been used as the standard against which new tests are compared. It is less subjective in reading and more sensitive than other tests.[36,37] Recently, an ELISA for IgG1 antibodies has been developed that is able to distinguish eosinophilic meningitis in patients caused by *A. cantonensis*.[43] Furthermore, the intrathecal synthesis pattern of IgG1+IgG2 and IgE can also contribute to the diagnosis of eosinophilic meningoencephalitis due to *A. cantonensis*.[44,45] Although the sensitivity of the ELISA tests may approach 100%, the instability of reagents and the need for sophisticated equipment are among the factors limiting their use in the field. A recently developed version of dot-ELISA has been reported for laboratory-infected rats with a sensitivity of 100%.[46] In humans, a dot-blot ELISA has shown promise in fulfilling the requirements of an economic and simple field test (Figure 2).[13,47—50]

As with any other nematode infection, the key to specific diagnosis is the use of an appropriate antigen. With crude extracted adult worm antigens or partially purified antigens, a considerable degree of false-positive reaction with other parasitic infections cannot be completely elimi-

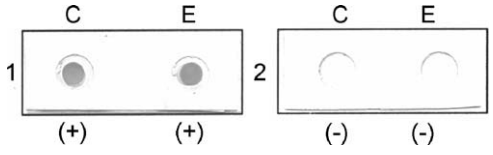

Figure 2 Dot-blot ELISA on nitrocellulose membrane for detection of specific antibodies in sera of patients using crude somatic extract (C) and electroeluted 31-kDa antigen (E) of *Angiostrongylus cantonensis*. 1: deeply colored dots show a positive reaction; 2: lightly colored or uncolored dots show a negative reaction.

nated.[11,12,16,51,52] In the past several years, progress has been made in the identification of the antigens that are specifically diagnostic for human angiostrongyliasis cantonensis. These include a 31-kDa glycoprotein antigen from the adult worm,[16,53,54] a 29-kDa antigen from the young adult worm,[55] and a 32-kDa protein obtained by elution from SDS-PAGE gels.[56] The differences in the estimated molecular weight of the specific antigens may perhaps be due in part to the parasite isolate/strain variability or technical differences in SDS-PAGE procedures and molecular weight calculations. There is no good reason at present to believe that these differences are real.

A 204-kDa young adult worm antigen purified by immunoaffinity chromatography has been reported to be a specific antigen.[57] More recently, a 104-kDa antigen has been demonstrated to be larva-specific and a 33-kDa antigen to be specific for the female adult worm.[58] In addition, differences among the subclasses of IgG in angiostrongyliasis cantonensis patients have been noted, with IgG4 directed primarily against a 29-kDa antigen.[59,60]

Most of the work to date on the various specific antigens recognized by immunoblotting has been on the 29-, 31-, or 32-kDa antigens. For simplicity the 31-kDa nomenclature is adopted here.[16] This antigen is among the principal antigens recognized by human angiostrongyliasis cantonensis sera as well as sera from immunized mice, rats, and rabbits.[16,61] Immunoblotting shows it to be of great potential for the immunodiagnosis of human angiostrongyliasis cantonensis.[16,53,54,61,62]

Partial purification of the specific 31-kDa antigen with gel filtration through Sephacryl S-200 has resulted in improved specificity.[63] When used in the ELISA to detect antibodies in the sera of patients with angiostrongyliasis cantonensis, the sensitivity reaches 100% and the specificity 98%. A more extensive purification procedure using electroelution from SDS-polyacrylamide gel, results in 100% diagnostic sensitivity and specificity on testing in ELISA.[64]

To date, immunoblotting has definitely improved antibody detection for routine diagnosis of human angiostrongyliasis cantonensis. At the Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University in Bangkok, Thailand, a standard ELISA using crude somatic antigens is used for routine screening, and all ELISA-positive specimens are tested by immunoblot for confirmation. A serum reacting with a specific 31-kDa band is indicative of *A. cantonensis* (Figure 3).

In recent years, substantial progress has been made in the development of a rapid diagnostic kit. A simple dot-blot ELISA with purified 31-kDa antigen has been developed for detecting *A. cantonensis* antibody, attaining 100% sensitivity and 100% specificity.[47,48] The dot-blot ELISA with purified antigen is as sensitive and nearly as specific as the immunoblots with a 31-kDa specific band.[13] Also, the test is much easier to perform than an immunoblot analysis. An in-house dot-ELISA kit with purified 31-kDa antigen has been evaluated to have an overall diagnostic sensitivity of 100% and specificity of 100% for human angiostrongyliasis cantonensis.[48] This dot-blot ELISA kit has performed well in a blinded multi-laboratory evaluation, without cross-reactions with sera of patients infected with other commonly occurring human parasites.[48] Furthermore, blood dried on filter paper has been used successfully, both in dot-blot ELISA and immunoblot.

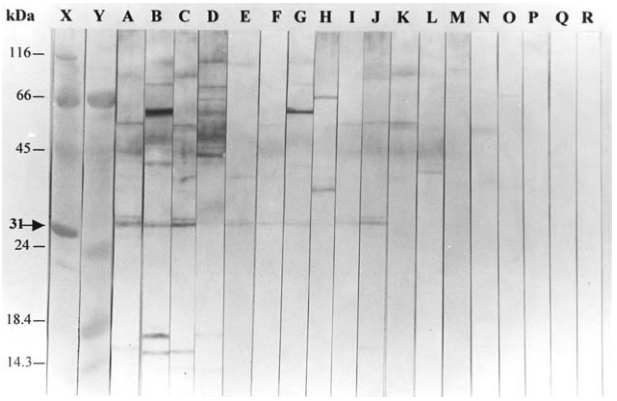

Figure 3 Immunoblots of serum samples of patients with angiostrongyliasis (A—J), gnathostomiasis (K), toxocariasis (L), filariasis (M), paragonimiasis (N), cysticercosis (O), and malaria (P) against crude extract of *Angiostrongylus cantonensis*. Q, R: normal control sera. Arrow indicates the 31-kDa band.

This diagnostic test kit has been used effectively for field studies in endemic areas, under the parasite control project conducted by the Department of Disease Control, Ministry of Public Health, Thailand.[49,50] The major difficulty in the wide-scale application of this simple and rapid test is the supply of adequate quantities of specific antigens. Perhaps recombinant protein could be the basis for a future diagnostic kit.

More recently, a multi-dot ELISA on a single nitrocellulose membrane strip (Figure 4) has been developed for the rapid and simple differential diagnosis of eosinophilic meningitis due to helminth infections using ultrafiltered, purified antigens of *A. cantonensis*, *Gnathostoma spinigerum*, and *T. solium* metacestodes, the most common parasites that invade the central nervous system and cause eosinophilic pleocytosis.[14] In this study, serum samples of 10 patients each with angiostrongyliasis cantonensis, gnathostomiasis, cysticercosis, toxocariasis, filariasis, paragonimiasis, and malaria as well as 10 healthy (control) subjects were investigated. Although there are weak cross-reactions among the parasite antigens of *A. cantonensis* and *G. spinigerum*, these do not interfere with judgment, as the darkest dot, which indicates the infecting parasite, is apparent in all cases. The advantage of this method is that semi-purified specific parasite antigens can be used with reliability. Further improvements using highly specific parasite antigens may make this multi-immunodot test more suitable for wide-scale use in field studies and diagnostic laboratories.

Although there have been only few studies on excretory/secretory (ES) antigens, the use of ES antigens from adult worms in an immunoblot test to detect a specific antibody of 31 kDa in patients with angiostrongyliasis cantonensis appears to be as good as the somatic extracts in terms of sensitivity and specificity.[65] However, ES antigens are not used for routine diagnosis because of difficulty in obtaining a substantial working quantity of the ES proteins for antigen preparation.

In addition, *Biomphalaria glabrata* (a snail intermediate host of *A. cantonensis*) shares some specific epitopes with *A. cantonensis*.[66,67] Two *B. glabrata* antigens with molecular weights of 48 kDa and 24 kDa react with the sera from angiostrongyliasis cantonensis patients but not with other

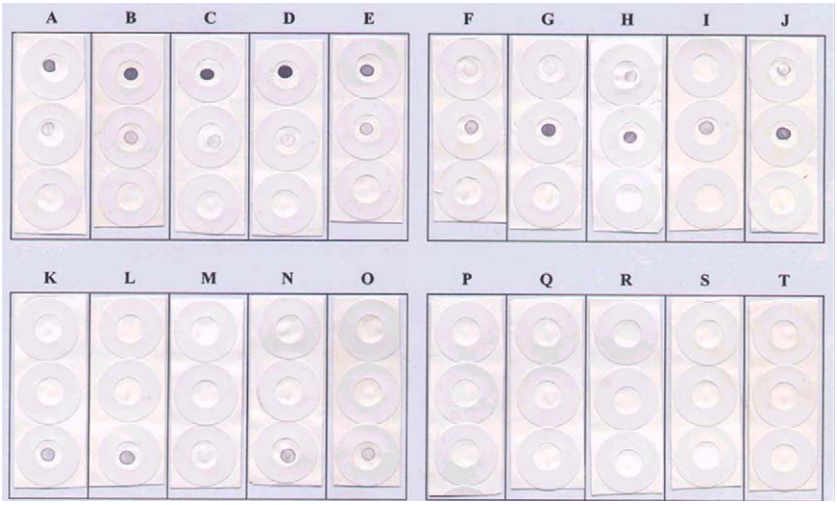

Figure 4 Multi-dot ELISA using ultra-filtered purified antigens of *Angiostrongylus cantonensis* (top dot), *Gnathostoma spinigerum* (middle dot), and *Taenia solium* metacestodes (bottom dot), on nitrocellulose membrane strip for detection of specific antibodies in sera of patients with angiostrongyliasis (A—E), gnathostomiasis (F—J), cysticercosis (K—O), toxocariasis (P), filariasis (Q), paragonimiasis (R), and malaria (S), and normal control serum (T). A deeply colored dot shows a positive reaction; a pale dot or no color indicates a negative reaction.

parasitic infections.[67] The possibility of using protein of *B. glabrata* snails as a source for generating specific *A. cantonensis* antigens needs to be explored.

Antigen detection

An alternative to antibody detection is the antigen detection assay, which demonstrates the presence of parasites in the hosts. In spite of the diagnostic improvements related to the detection of antibody techniques, the demonstration of specific *A. cantonensis* antigens in the serum and CSF will be a valuable addition to testing options. The detection of antigens provides a more rapid confirmation of acute or active infection.

Circulating serum antigens can be detected in humans with angiostrongyliasis cantonensis. To detect defined *A. cantonensis* antigens in patient blood during acute infection, alternative antigen capture assays have employed monoclonal antibodies directed against parasite-specific antigen with relatively high specificity and reasonably good sensitivity.[16,62,68—73]

Several laboratories have produced panels of monoclonal antibodies that are reactive toward the somatic extract antigens of *A. cantonensis* — e.g., several monoclonal antibodies against the adult worm antigens[74] and four specific IgG monoclonals against the young adult worm antigens.[75] However, they show low specificity and sensitivity.[76]

In more recent years, specific monoclonal antibodies to *A. cantonensis* have been produced for clinical diagnosis of active angiostrongyliasis cantonensis.[16] The AW-3C2 monoclonal antibody has been used as a capture reagent in sandwich ELISA to detect a specific circulating antigen in the sera of angiostrongyliasis cantonensis patients with 100% specificity and 50% sensitivity.[16,69,70] Very promising results have been achieved using double monoclonal antibodies (AcJ1 and AcJ20) with a specificity of 100% in serum and CSF from patients with eosinophilic meningitis or meningoencephalitis with worms recovered from them.[68] Recently, three specific monoclonals (2A2, 3F1, 4H2) against the adult worm have shown a positive detection rate of 86.4%.[72,73]

For antigen detection, immunodot, a rapid and simple test, has also been developed using specific *A. cantonensis* monoclonal antibody (AW-3C2).[62] This approach is also promising in terms of possible diagnostic test kits. Although the diagnostic specificity approaches 100%, the sensitivity is around 60%.[62]

As low sensitivity of the tests may arise with the binding of a single antibody to a single antigenic epitope, using a panel of monoclonals that belong to different subclasses of IgG to react with different epitopes on the same circulating antigen may overcome the problem of sensitivity.[68] A more sensitive method for antigen detection, the immuno-PCR, detects a circulating 204-kDa AcL5 antigen in human patients with eosinophilic meningitis or meningoencephalitis with 100% specificity and 98% sensitivity.[77]

Concluding comments

As clinical symptoms are not solely diagnostic of helminth infections that cause eosinophilic meningitis, definitive diagnosis of individual infections is required for prompt, appropriate, and adequate treatment. In the absence of parasite recovery, the diagnosis of angiostrongyliasis due to *A. cantonensis* has recently been achieved by immunological approaches through antibody as well as antigen detection.

Difficulties with the supply of adequate quantities of purified specific antigen for immunodiagnostic use can perhaps be overcome by the use of antigen expressed *in vitro* through recombinant DNA techniques. The dot-blot ELISA, which does not require specific equipment, and from which positive reactions can be observed with the naked eye with reliability, may make the test more appropriate and econom-

ical in developing countries. An alternative to immunodiagnosis is DNA probe-based diagnosis. This method has the advantage of a direct assay of current infection. A PCR-technique has been described for the DNA detection of abdominal angiostrongyliasis due to *Angiostrongylus* (= *Parastrongylus*) *costaricensis* in clinical samples in Brazil.[78] As the primers were based on *A. cantonensis*, a similar application may be possible in the case of *A. cantonensis* infection.

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